THE POTENTIAL OF CITRIC ACID PRODUCTION USING PINEAPPLE WASTE

Lee Ting Hun[1] **Hanapi Bin Mat**[2]
[1]Department of Bioprocess Engineering [2]Department of Chemical Engineering
Faculty of Chemical Engineering and Natural Resources Engineering
Universiti Teknologi Malaysia, Skudai, Johore, Malaysia.
E-mail: ctlee@fkkksa.utm.my

ABSTRACT

Citric acid fermentation with *Aspergillus niger* ATCC 10577 in stirred tank reactors (5 l) were carried out using pineapple solid and liquid waste. Highest yield (13.07 g/l) was obtained with the solid pineapple waste while liquid pineapple waste obtained a 6.59 g/l yield based on the sugar consumed. Shake flasks culture were done initially to find the suitable pH and dilution rate.

Keywords: Citric acid, pineapple liquid waste, pineapple solid waste, *Aspergillus niger*

INTRODUCTION

Environmental pollution by wastes generated from economic activities such as chemical, petrochemical, agricultural and food industries are common examples and problems in the world nowadays. Often, due to stringent environmental regulation regarding to waste disposal, the industry have to provide proper treatment system which require investment. This might result an increase in production cost and so on company competitiveness. However, if wastes can be transformed into valuable products such as organic acid, thus would heightened the profit and competitiveness of the industry.

Base on the year 1994, the amount of waste being produced from the pineapple canning factory was 11750 tones (MPIB, 1995). With this figure, it has more than enough carbon source to produce organic acid. In order to do so, the waste was used as a carbon source in submerged fermentation by *Aspergillus niger* ATCC 10577 to produce citric acid.

In short, this study is an attempt to use the pineapple waste to produce citric acid by using Aspergillus sp. which the results will definitely benefit to the industry. Before the fermentations were carried out, the shake flasks culture were done in order to obtained the suitable pH and dilution content for the growth of the strain.

MATERIALS AND METHODS

Chemical Analysis

The citric acid content was measured by the Wasters HPLC machine and its subsidiary equipment. The column used was Waters, Nova-Pak C_{18}. The sucrose, glucose and fructose were measured by the Wasters HPLC machine and its subsidiary equipment. The column used was Whatman, Partisil 10 ODS Carbohydrate.

Fermentation Experiment

Fermentation experiments were carried out in a stirred tank reactor using 5 liter glass vessel (Biostat B) with a working volume of 3 liter. Aeration was be 1 vvm throughout the fermentation. The agitation speed was maintained at 300 rpm. The temperature was controlled at 30°C and the pH was continuously recorded but not controlled. The initial pH used was 4.0. Methanol was added to a final concentration of 4% (v/v) at the beginning of fermentation. The inoculum (10% v/v) was a 24 hour of shake flask culture.

Strain

Aspergillus niger ATCC 10577 obtained from German Collection of Microorganisms and Cell Cultures (DSM).

Carbon Source

The liquid and solid pineapple waste were obtained from Lee Pineapple Pt. Ltd. The solid waste was dried at 52°C for 60 hours then ground to the size of not more than 25 mm^2 (control by a 5 x 5 mm iron filter) and stored in 4°C (Modified from Tran and Mitchell, 1995). These carbon sources were change monthly.

Inoculum Media

Precultures were performed in 500 ml Erlenmeyer flasks containing 300 ml of medium (synthetic). 1 ml of spore suspension in distilled water (approximately 10^8 spores/cm^3) was used as an inoculum and the flasks were incubated at 30°C on a shaking incubator at 200 rpm for 24 hours. The synthetic medium as suggested by Kong et al. (1995) was used in the experiment which contained (g/l): sucrose, 100; NH_4NO_3, 1; $MgSO_4$, 0.25. The pH of the medium was adjusted to pH 3.8 by using 1 M HCL.

Shake Flask Culture

Shake flask culture was done in a 500 ml Erlenmeyer flasks containing 300 ml of medium. 1 ml of spore suspension in distilled water (approximately 10^8 spores/cm^3) and nutrient such as NH_4NO_3 (1 g/l) and $MgSO_4$ (0.25 g/l) was added and the flasks were incubated at 30°C on shaking incubator at 200 rpm for 24 hours.

RESULTS AND DISCUSSION

Shake Flask Calture

Several parameters were examined for their suitability on the growth of *A. niger* ATCC 10577 in the shake flask experiment.

Effect Of Initial pH By Using Synthetic Medium (SF1). The optimum initial pH was found to be around 4.0 (Table 1) when synthetic medium was used to test the suitable initial pH for the growth of the strain used.

Table 1: Effect of initial pH by using synthetic medium

pH	Growth
2.0	-
2.5	-
3.0	++
3.5	+++
4.0	+++

(-) No growth
(+) Growth
(++) Good Growth
(+++) Excellent Growth

Effect Of Initial pH By Using Pineapple Waste Juice (SF2). The pineapple waste juice with different initial pH did not effect the growth of the strain (Table 2).

Table 2: Effect of initial pH by using pineapple juice

pH	Growth
2.0	-
2.5	-
3.0	-
3.5	-
4.0	-

Effect Of Dilution Of H_2O With Pineapple Waste Juice (SF3). The best grown was observed in the 1:9 (pineapple waste juice:H_2O) dilution. However the growth was still possible on the 5:5 dilution. There was no growth being observed at all in the 3:1 dilution (Table 3).

Table 3: Effect of dilution of H_2O with pineapple waste juice

Dilution (P. W. Juice: H_2O)	Growth
1:9	+++
1:1	+
3:1	-

From the SF1 results, it is clearly stated that the initial pH influenced the growth of *A. niger*. The suitable pH was found to be around 4.0. This result is agreed by Xu et al. (1989). However, the pH has no effect on the growth when pineapple liquid waste was used. This showed that the key parameter was not pH effecting the growth. The SF3 was carried out to find the suitable carbon source dilution rate. The suitable dilution rate was found to be 1:9 (pineapple liquid waste: H_2O). This may be due to the high concentration of BOD_5 , COD and metals ions (Table 3) that ceased the growth of *A. Niger*.

Fermentation

Citric Acid Production Using Solid Pineapple Waste (S1). With the proportion of 1:9 (Solid pineapple waste:H_2O), and the assumption of the specific gravity of pineapple waste is same as the water. The citric acid content increased gradually from the very beginning of fermentation till the highest point of 1.15 g/l at the 4th day. At the initial stage of fermentation, it was found that the inoculum and the pineapple waste contained 0.21 g/l of citric acid totally. Therefore the net production of citric acid from pineapple solid waste would be 0.94 g/l. The yield was found to be 0.35 g/l from 100 g of pineapple waste. The total yield based on the sugar consumed would be 13.07%.

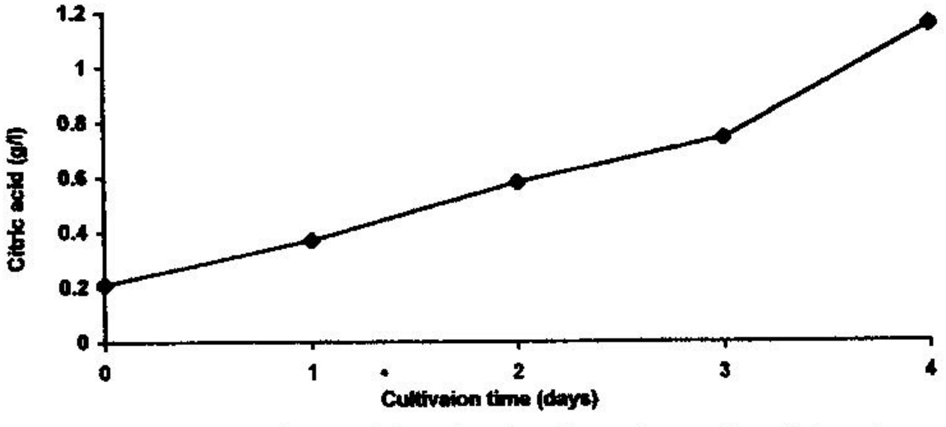

Figure 1: Citric acid production from pineapple solid waste

The sucrose, glucose and fructose concentration decreased gradually since the initial stage of fermentation. The sucrose was exhausted at the 2nd day of fermentation which is 3.27 g/l and the concentration of glucose was exhausted at the 3rd day of fermentation which is 1.63 g/l. The fructose was totally consumed at the 4th day with the concentration of 2.29 g/l.

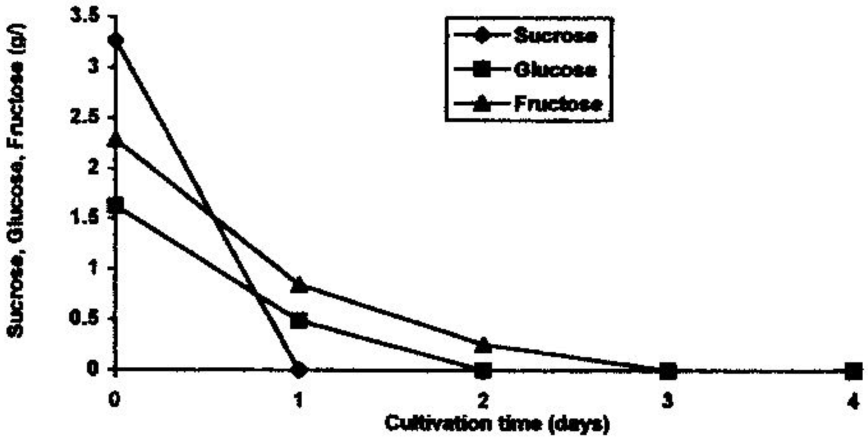

Figure 2: Sucrose, glucose and fructose consumption in solid waste

__Citric Acid Production Using Pineapple Liquid Waste (L1).__ Dilution of 1:9 (Pineapple Liquid Waste:H_2O), with the assumption of the specific gravity of pineapple waste is same as the water. The citric acid content increased gradually to the highest production of citric acid obtained at 0.73 g/l at the 4th day of fermentation. At the starting point of fermentation, the total citric acid from inoculum media and pineapple waste was 0.29 g/l. Therefore, the net production of citric acid from pineapple liquid waste was 0.44 g/l. The yield was found to be 0.16 g/l from 100 ml of pineapple liquid waste. The total yield based on the sugar consumed would be 6.59%.

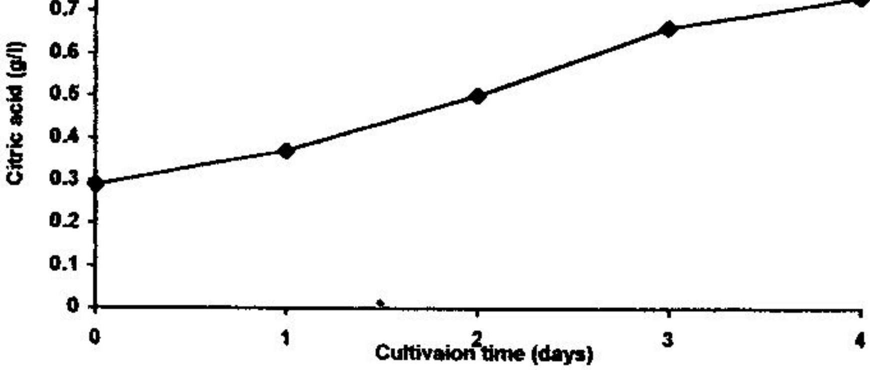

Figure 3: Citric acid production from pineapple liquid waste

All these three sugars decreased gradually starting from the very beginning of fermentation. The total amount of sucrose consume was 2.42 g/l at the 2nd day of fermentation and the concentration of glucose was exhausted at the 3rd of fermentation with the amount of 2.09 g/l. The fructose was gradually reduced and totally consumed at the 4th day of fermentation with the concentration of 2.17 g/l.

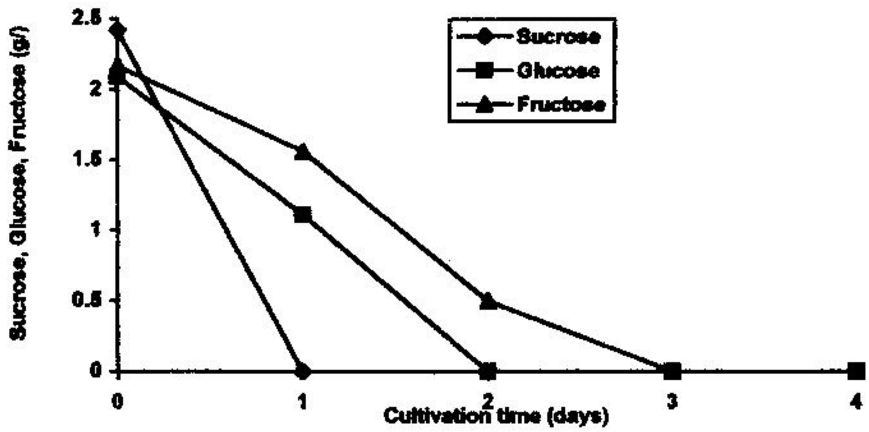

Figure 4: Sucrose, glucose and fructose consumption in liquid waste

Fermentation of solid and liquid pineapple waste using *A. niger* ATCC 10577 has revealed the possibility of the production of citric acid. The citric acid reached 1.15 g/l and 0.73 g/l in pineapple solid and liquid waste respectively at the end of 4th day. The citric acid produced from pineapple solid waste was higher compare to pineapple liquid waste (0.5 g/l higher). The yield of solid waste is 6.48% higher than the liquid waste based on the sugar consumed. Therefore, it is clearly showed that the solid waste is more productive.

Although the sucrose, glucose and fructose concentration exhausted at the third day of fermentation while the concentration of citric acid still rising. This may be due to other sugar source such as galactose, maltose and other sugar that present in the waste but with a very low concentration.

From Figure 2 and 3, the sugar consumption trend has shown that the more favorable carbon source is sucrose followed by glucose and then fructose. This is further agreed by Hossain et al. (1984).

CONCLUSION

Submerged fermentation of pineapple waste for solid and liquid produced 1.15 g/l and 0.73 g/l of citric acid respectively at the 4th day of fermentation. It is clearly shown that the solid waste is more productive than the liquid waste. It is also showed that the growth of A. niger ATCC 10577 in 100% liquid waste is not possible from the shake flask experiments. However with the recommended dilution rate, production may be carried on.

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